

## Pattern and Ontogeny of the Foliar Venation of *Bobea elatior* (Rubiaceae)

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BEFORE A FULL UNDERSTANDING and appreciation of the various patterns of foliar venation can be attained there must be a greater knowledge of the ontogenetic processes which result in the diverse patterns of veins. The ontogenetic investigations to date suggest that there is a correlation between the shape and arrangement of the plate-meristem cells of the young leaf which produces the minor vein system and the pattern of the mature venation. In the case of the prevalent pattern in dicotyledons of polygonal areoles as exemplified by *Liriodendron* (Pray, 1954, 1955a), the plate meristem concerned is composed of small isodiametric cells whose planes of anticlinal division (with respect to the surface) are random; i.e., they are not oriented in any particular direction. The veins which comprise the mature minor venation are similarly disposed. In *Hosta* (Pray, 1955b, c), a monocotyledon, on the other hand, the intercostal venation (i.e., that between the primary veins), whose course is roughly at right angles to the course of the primaries, is derived from a plate of cells elongated at right angles to the primaries. Likewise, the study of Foster (1950, 1952) on the distinctive foliar venation of *Quiina* shows that the lineolate minor venation is derived from similarly oriented elongate cells in the plate meristem of the developing leaf.

The present study was initiated therefore to examine the ontogeny of another distinctive pattern of foliar venation as displayed by the leaf of *Bobea elatior*. The genus *Bobea* with five species is endemic to the Hawaiian Islands. An extensive survey (Pray, 1953: 172-264) of the tribe of the Rubiaceae in which it is placed, the Guettardeae (composed entirely

of woody trees and shrubs), has revealed an astonishing variety of foliar venation patterns which exhibit varying degrees of expression of a lineolate disposition of the minor veins. The simpler patterns present in the Guettardeae appear to represent initial phases in the evolution of a markedly lineolate pattern of minor venation. *Bobea*, as a representative of those genera which display a lineolate pattern to a slight degree only, is of particular interest in broadening our understanding of variation in foliar venation and the ontogenetic processes which lead to such variation.

### MATERIALS AND METHODS

The writer is indebted to Dr. Sherwin Carlquist for providing the material which formed the basis for the present study. The material was collected on the Palolo-Mt. Olympus trail on the island of Oahu, Territory of Hawaii. Vegetative buds and leaves in various stages of development were preserved in FPA. A voucher specimen of the same material has been deposited in the Herbarium of the University of California, Berkeley (Carlquist H6, August 1953). Mature leaves and those in several stages of development were cleared with 2½ per cent NaOH to facilitate the study of the overall venation pattern. Sections were made at 7 and 8  $\mu$ , with a great predominance of paradermal sections which have been found to be especially important in ontogenetic studies of venation patterns. All sections were stained with tannic acid—ferric chloride—safranin with a weak solution of fast green used to further differentiate the safranin.

### VENATION PATTERN

The leaf blade of *Bobea elatior* is broadly lanceolate and varies from 4.5 to 11 cm. in length and 2 to 4.5 cm. in width. As is true

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of all members of the Guettardeae (indeed, of almost all Rubiaceae), the major venation of the leaf consists of a midrib with a pinnate series of secondary veins arranged in a camp-todromous manner (Ettinghausen, 1861: xvi); that is, the extremities of the secondaries curve acropetally near the leaf margin. In addition to the secondaries there are other prominent but smaller veins which diverge from the midrib and extend toward the margins. These intermediate veins (Foster, 1950: 163) are, however, entirely enclosed within the panels or areas delimited by the secondary veins. Such areas will henceforth be referred to as intersecondary or intercostal panels. In actuality the distinction between some intermediate veins and strong tertiaries is arbitrary because the two do intergrade.

In the mature leaf, tertiary veins and veins of lesser categories are not readily distinguishable. Hence it is convenient to refer to the entire intercostal venation exclusive of the obvious intermediates as the minor venation. It is the pattern, histology, and ontogeny of the minor venation with which the present study is particularly concerned. In a cleared leaf (Fig. 1) the minor veins, except for occasional obvious tertiaries, are fairly uniform in size and relative prominence. The ultimate areoles (smallest units of mesophyll completely enclosed by veins) are delimited for the most part by veins of the fifth and sixth orders. However, for the above mentioned reason, vein categories within the system of the minor venation will not be considered in descriptions to follow.

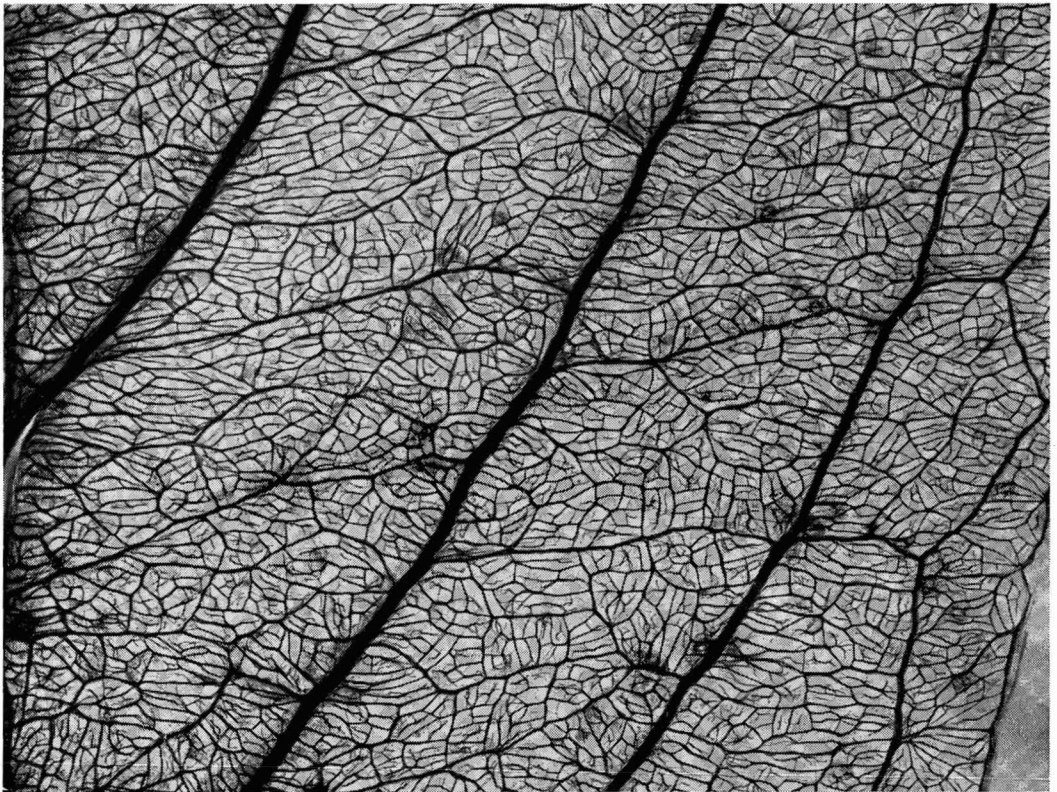


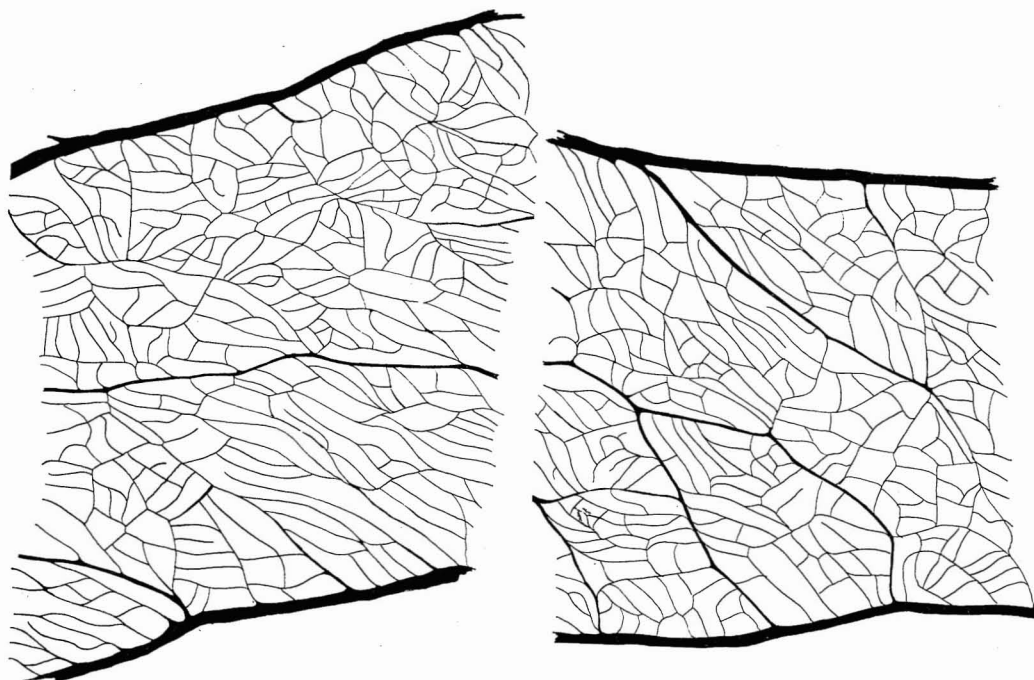
FIG. 1. Portion of a cleared lamina showing the general nature of the mature venation,  $\times 7$ . Midrib at left; several secondary veins in part extend diagonally toward upper right.

The ultimate areoles of *Bobea* display a marked tendency to be rectangular and often distinctly elongated. While actual areole shape is extremely variable the tendency toward rectangularity is well enough expressed to give the minor venation a distinctive character which is readily distinguishable from the minor venation of such a leaf as that of *Liriodendron* and many other dicotyledons with their polygonal areoles. The tendency of the minor venation to be made up of elongated areoles is interpreted as a weak expression of the lineolate type of venation which becomes highly developed in some members of the tribe Guettardeae (Pray, 1953: 174–233). While the cleared leaf at low magnifications (as in Fig. 1) displays quite obviously the general character of the minor venation, the abundant sclerenchyma in the blade does obscure the details, particularly as regards the occurrence and nature of vein endings. There

are fibers associated with every vein. In transverse section (Fig. 6) the fibers, which are located adaxial to the tracheary elements, are shown to comprise in fact the greater part of the vascular bundles. Strands of fibers and individual fiber cells extend from the vascular bundles into the mesophyll, in the region within the palisade layer, and between the palisade and hypodermal layers (Figs. 2, 3, 6). Careful study of cleared leaves at higher magnifications (Figs. 2, 3) shows that structures appearing to be vein endings are actually devoid of conductive tissues as such and consist of sclerenchyma only. Thus drawings of the true conductive system (Figs. 4, 5) show that most of the areoles lack vein endings. The latter are rather rare, in fact, in the present material. Apparently areoles without freely terminating vein endings are infrequent in the foliar venation patterns of dicotyledons in general (Esau, 1953: 424).



FIGS. 2 (left), 3. Small areas of the cleared lamina showing the nature of the ultimate areoles,  $\times 65$ . Note fibers extending from the veins into the mesophyll. Arrow in Figure 2 indicates an idioblastic sclerenchymatous element.



FIGS. 4, 5. Drawings of portions of two intersecondary panels (secondary vein at top and bottom of each figure),  $\times 10$ . Note scarcity of free-terminating vein endings.

#### LEAF HISTOLOGY

The general arrangement of tissues of the lamina of *Bobea* is distinctive enough to merit comment in this account. The epidermis is uniseriate on both leaf surfaces with the stomata limited to the lower. Beneath the upper epidermis there is a two-layered hypodermis (Fig. 6) of rather large, closely-packed cells apparently lacking chloroplasts. Ontogenetically, the hypodermis is derived from a single ground meristem layer. The latter is the adaxial subprotodermal layer which in many leaves produces the palisade layer of the mesophyll or its equivalent. The systematic significance, if any, of the hypodermis in the Rubiaceae is not known. It does occur in the only other species of *Bobea* (*B. timonioides*) available for study and in at least one species of *Timonius*, a closely related genus, but has not thus far been found in other genera of the tribe (Pray, unpublished). Solereder (1908: 445) and Metcalfe and Chalk (1950: 761)

record the sporadic occurrence of this feature in several other genera of the family. The palisade layer, which is nearly medially situated in the leaf, is biseriate for the most part. The spongy layer is quite loosely arranged, with the individual cells of rather irregular form.

The smaller vascular bundles which constitute the minor venation extend from the lower limits of the hypodermis into the spongy mesophyll, with the conductive tissues located within the latter. As noted above, the greater part of the vascular bundle is sclerenchymatous, with individual fibers and fiber strands extending beyond the limits of the conductive tissues. There are rarely idioblastic sclerenchymatous elements in the areoles (Fig. 2, center). These often appear to be intermediate between typical fibers and elongate foliar sclereids, such as certain of the unbranched types described in *Trochodendron* (Foster, 1945: pl. IV).



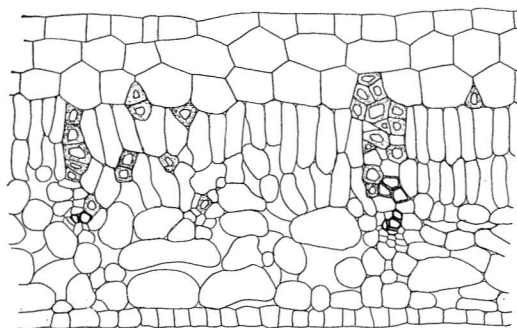


FIG. 6. Transverse section of mature lamina showing general leaf histology,  $\times 200$ . Tracheary elements indicated with black walls; sclerenchyma with stippled walls.

#### ONTOGENY OF THE VENATION

Throughout blade ontogeny a basipetal pattern of maturation prevails, both in the initiation of secondary veins and in the differentiation of the minor venation between the secondaries. When the intersecondary panels are first delimited the cells of the plate meristem which will produce the minor venation are essentially isodiametric (Figs. 8, 17). The insertion of new cell walls anticlinal to the surface during this phase is apparently random. In a panel of such isodiametric cells, localized, oriented divisions in a continuous series of cells produce the first procambial strands (tertiaries) (Fig. 9) in a manner similar to that described for *Liriodendron* (Pray, 1955a: 21). Concurrent with the differentiation of the tertiary procambial strands the nature of the intervening ground meristem becomes noticeably altered with the establishment of a general tendency for the cells to become markedly longer than wide (Figs. 7, 10, 13). This condition is due largely to repeated cytokinesis of a given meristem cell in the same plane producing small packets of similarly elongated cells (Figs. 10–14). Similar divisions in two or possibly three contiguous cells can produce the same effect. The latter apparently happens infrequently because there is a decided tendency for the planes of cell division in adjacent cells to be completely unrelated and, in fact, they are

rather frequently more or less perpendicular to one another. The tendency for small parallel groups of ground meristem cells to be formed, each independently oriented with respect to their neighbors, is characteristic of the ground meristem during the phases of leaf development concurrent with the formation of the minor venation as illustrated by examples in Figures 10–13. In a given section (Figs. 10–13) elongate cells are not evenly distributed. Sometimes small areas will display considerable regularity while others of the same leaf will have a rather sporadic expression of this tendency. It is from such parallel groups of cells that series of similarly oriented procambial strands are delimited (Fig. 16). Thus the essential nature of the minor venation of *Bobea* is determined by planes of cell division in the ground meristem immediately preceding procambial differentiation.

In the development of the minor venation (exclusive of obvious tertiaries) the delimitation of the procambium from the ground meristem appears to follow a rather orderly procedure when studied in paradermal section. A series of elongate cells derivable from subdivisions of a single cell or several adjacent cells is formed in this process. Most of these will subsequently redivide perpendicularly to the previously predominant plane of division, while one or sometimes several remain undivided. The elongate cells thus delimited are precursors of procambial strands. This series of steps can be illustrated by the following figures. In Figure 11 (top) there is a group of cells elongated perpendicularly to the course of the two procambial strands delimiting the areole. If, then, two or more of these cells remain undivided while the intervening ones further subdivide by a series of divisions at right angles to their long axes, the initials of procambial strands separated by a group of nearly isodiametric cells which are potentially ground tissue will be delimited. This apparently has occurred in Figures 12 (upper left) and 14 (upper right). This same

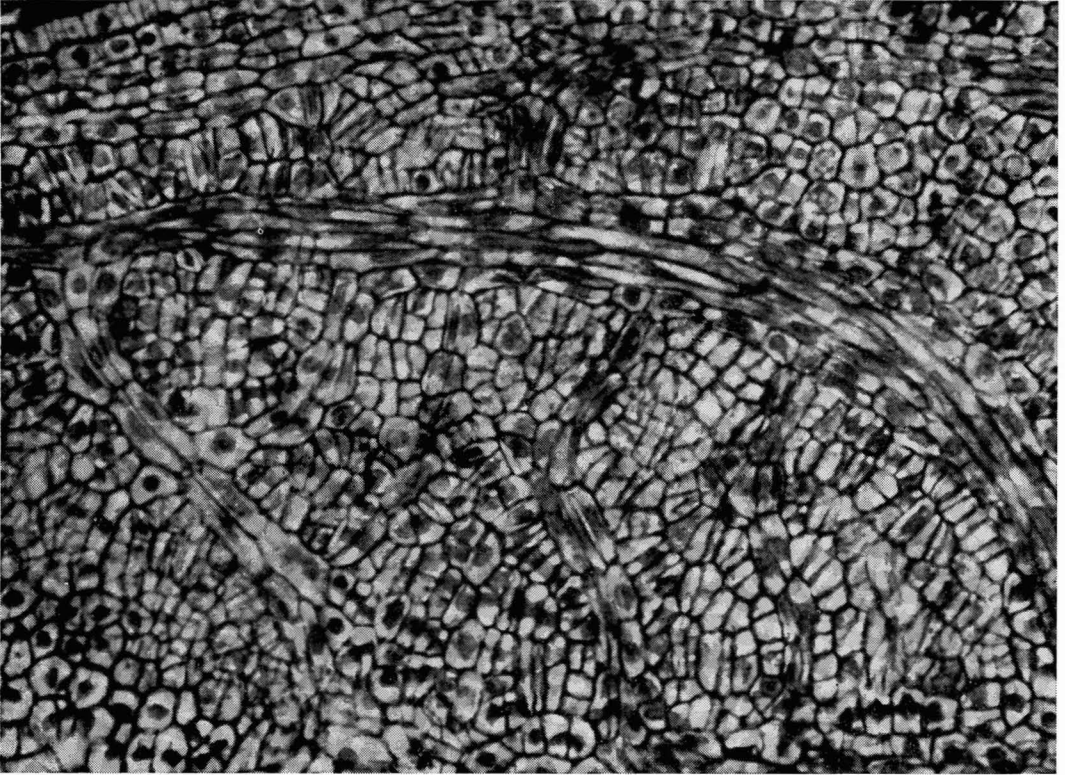


FIG. 7. Paradermal section of very young lamina showing about one half of an intersecondary panel with differentiating tertiaries,  $\times 725$ . Leaf midrib at lower edge; leaf margin at top. Prominent procambial strand extending from right to left is a secondary vein.

process has progressed farther in Figure 16. Thus areoles are produced which in the mature leaf often occur in more or less parallel series (see also Fig. 21).

During the differentiation of much of the minor venation, particularly below the quaternary category, there is a predominance of strands which are initially single celled (as seen in paradermal section). This is particularly true where a series of similar areoles have been delimited in a precise geometric manner

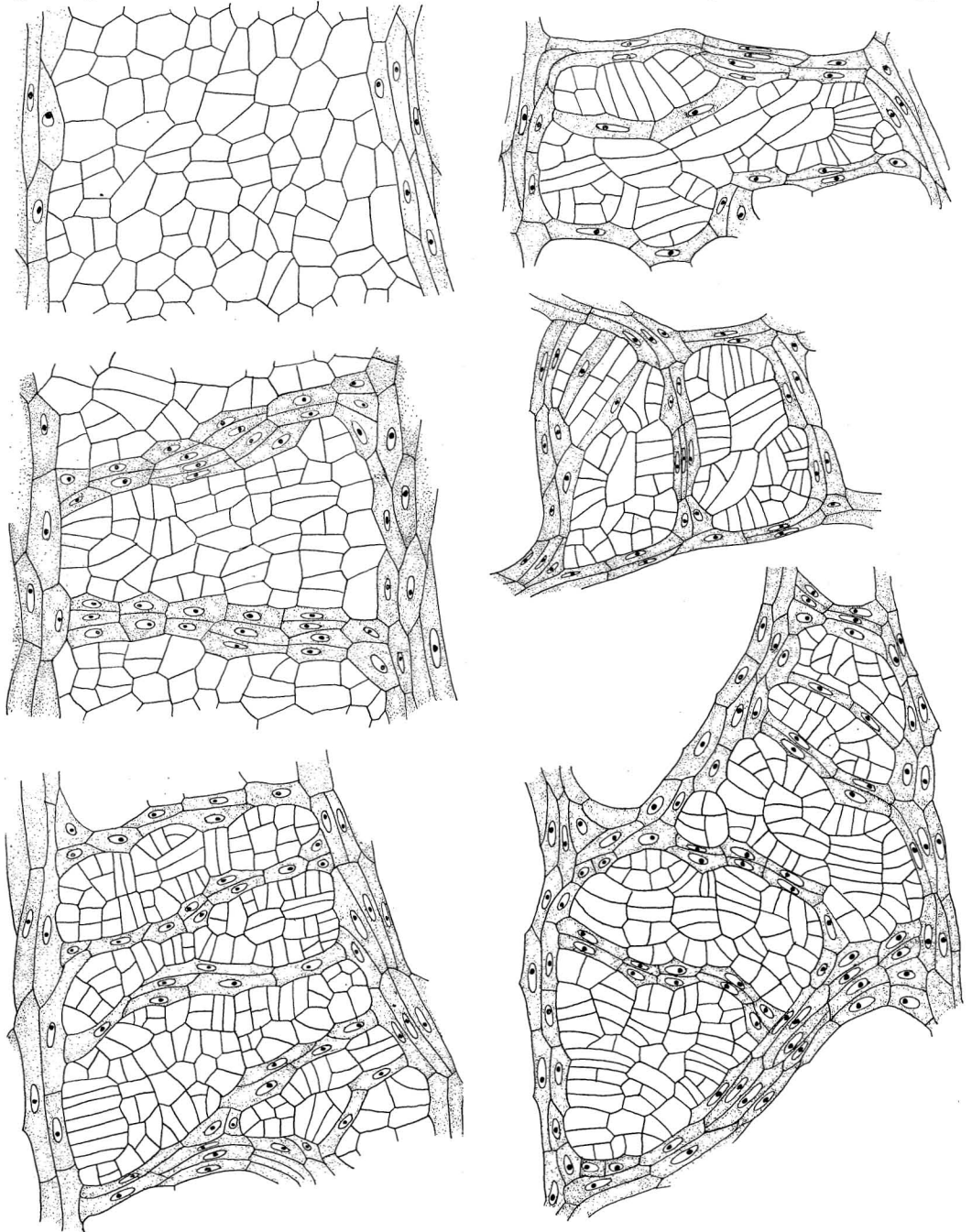
as described above (Figs. 13–15). Most often, on the other hand, strands which apparently were initially more than one cell in length are curved (Fig. 16). Also included in this category are those forked strands (Fig. 15, upper half) which delimited areoles of various irregular perimeters.

Much of the minor venation of *Bobea* shows less parallel orientation of veins than the preceding account suggests. This is directly attributable to the fact that much of the original

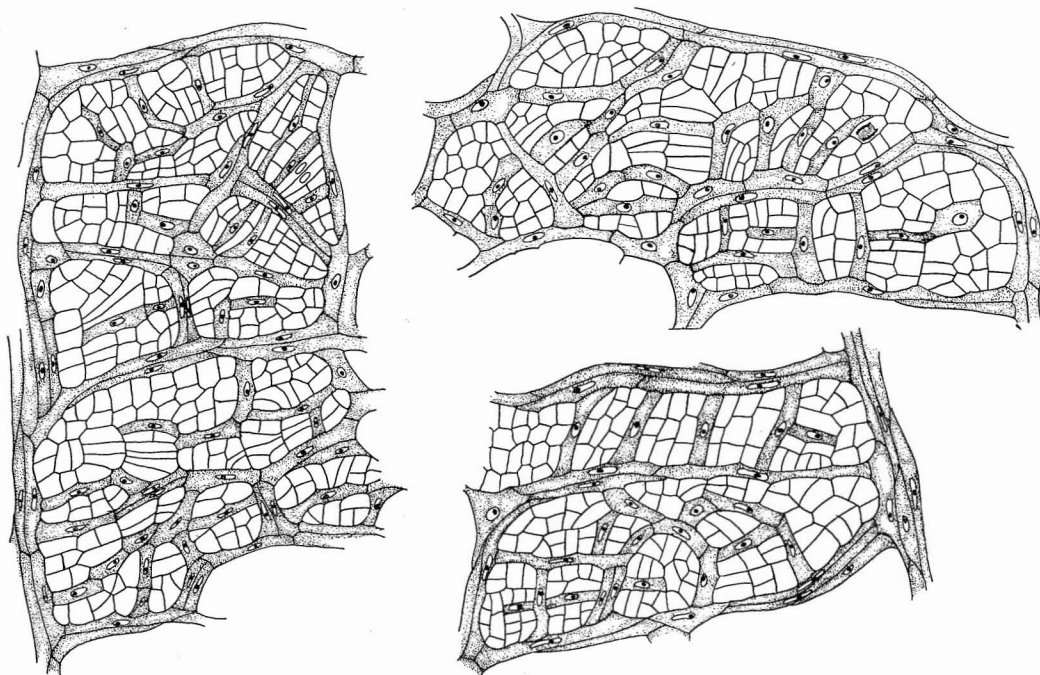
FIGS. 8–13. Portions of paradermal sections illustrating stages in the initiation and early ontogeny of the minor venation,  $\times 700$  (except Fig. 8,  $\times 1000$ ). Figure 8 (*upper left*): Intersecondary panel prior to appearance of any intersecondary procambium. Figure 9 (*middle left*): Similar panel with differentiated tertiary procambial strands. Note change in shape of ground meristem cells as compared with preceding figure. Figure 10 (*lower left*): Panel similar to Figure 9 in which subdivision of the ground meristem has proceeded further. Transverse procambial strands are tertiaries. Figures 11 (*upper right*) and 12 (*middle right*): Similar small areas illustrating the general nature of the ground meristem at the time quaternaries are being delimited. Figure 13 (*lower right*): Portion of intersecondary panel (midrib at left) with tertiary procambial strands. Another portion of the same panel is shown photographically by Figure 7.

ground meristem does not have the degree of precise parallel subdivision described in the foregoing ontogenetic series. However, the right-angled intersection of most veins and

the general rectangular nature of the ultimate areoles is related to the tendency for veins and intervening panels of mesophyll to be derived from rectangular initials. The appar-



FIGURES 8-13



FIGS. 14-16. Portions of paradermal sections in which ultimate areoles are probably being delimited (or have been delimited),  $\times 500$ . Figure 14<sup>1</sup> (left): Secondary vein at left margin; other large veins in these figures are tertiaries and quaternaries. (Fig. 15, *top right*; Fig. 16, *lower right*.)

ent random spatial relationship among various small groups of minor veins is a result of the apparent random planes of cytokinesis in each original ground meristem cell as compared with its neighbors.

A comparison of transverse sections in successive stages of leaf ontogeny (Figs. 17, 18) demonstrates that the sclerenchyma and vascular tissues of a vein have a common origin from an original procambial initial by a series of longitudinal divisions periclinal to the leaf surface. The lowermost cell or several cells then subdivide in various longitudinal planes to produce a strand of narrow procambial cells. During the earlier phases of differentiation these two components of the fibrovascular system of the leaf are not readily distinguishable in paradermal section. Therefore, it is only in the later phases of leaf development that we can distinguish clearly the future sclerenchyma from the actual conductive tissues. Figures 19 and 20 show the

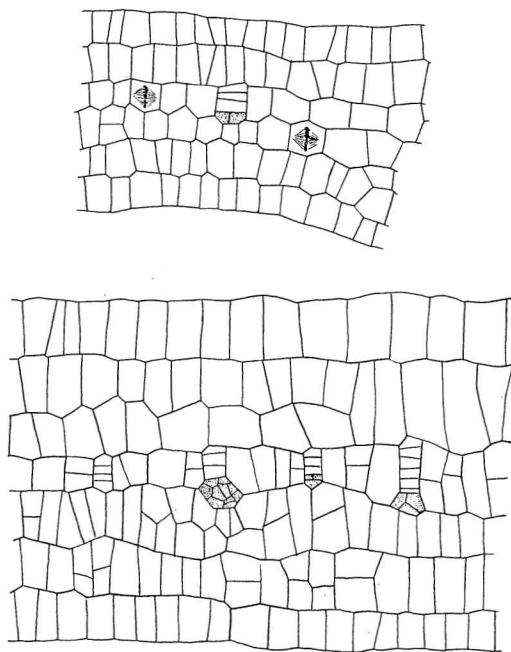
same area of a lamina at two levels; Figure 20 shows a section  $12\ \mu$  closer to the upper surface than Figure 19. In Figure 19 there are shown a number of probable ultimate areoles delimited by obvious procambial strands, forming a pattern characteristic of the mature venation of the species. At this level in the relatively immature lamina (15 mm. long) the plate meristem has apparently been fully segregated into procambium and fundamental tissue with a reversion to a completely random insertion of anticlinal walls producing groups of more or less isodiametric cells (*viz.*, Fig. 8). Apparently no more procambial strands are to be formed at this level. Just  $12\ \mu$  above the latter section we find the pattern much more intricate and apparently still in the process of differentiation. The cells delimited from the ground tissue in Figure 20 are the initials of the sclerenchyma. Careful examination of the figures under discussion reveals that in addition to a series of



initials being present above each procambial strand there are also at the higher level numerous additional anastomoses. The latter mature into sclerenchyma. Also noteworthy in Figure 20 are the several initials which end freely in the ground tissue. These are the precursors of the abundant fibers which extend into the mesophyll in the mature lamina. Quite infrequently idioblastic cells (Fig. 20) are encountered which are presumably the initials of the occasional idioblastic fibers or fibro-sclereids which occur in the leaf of *Bobea*. Finally it may be noted that the ground tissue at the level of Figure 20 appears to retain a greater predominance of rectangular cells, suggesting that cell division is still active with the possibility of further differentiation of sclerenchyma continuing later than at the level of the clearly distinguishable procambium. The continued meristematic activity of this region is understandable in view of the late maturation of the palisade typical of dicotyledonous leaves in general.

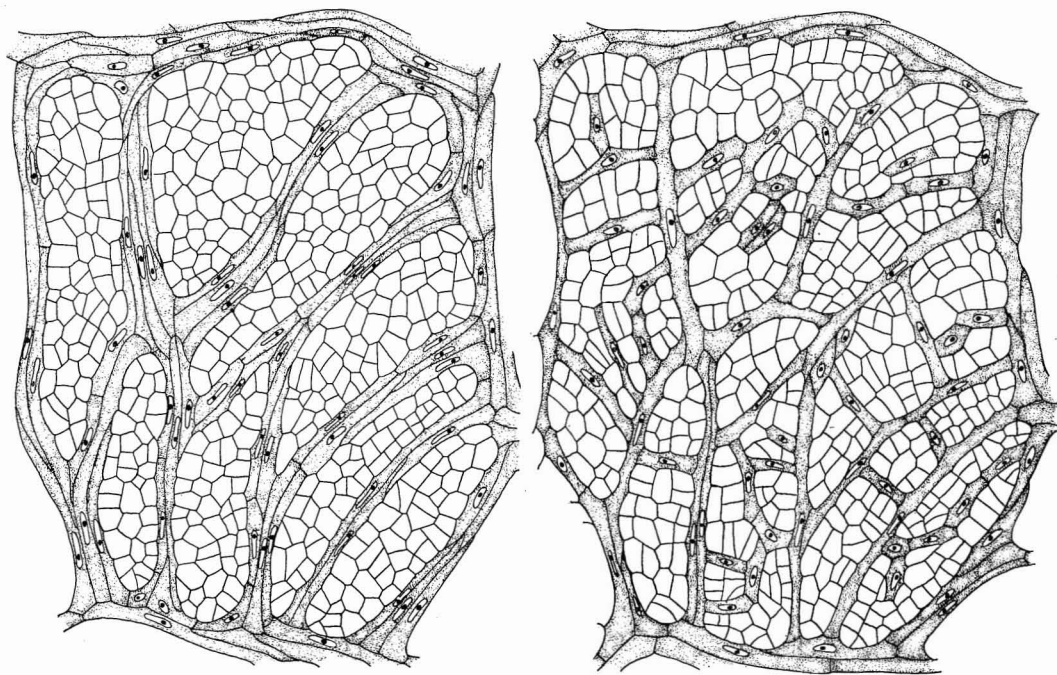
#### DISCUSSION

The older literature pertaining to the ontogenetic aspects of foliar venation and the modern histogenetic accounts have been reviewed and discussed by Foster (1952: 752–755) and Pray (1955c: 701–706). The present account of the leaf of *Bobea* appears to substantiate the ontogenetic pattern suggested in the latter paper. Namely, there is a definite correlation between the shape and arrangement of the plate meristem cells which gives rise to the vein system and the venation pattern of the mature leaf. In fact, a remarkable similarity will be found when the pattern of cell shapes in the ground meristem (Fig. 13) is compared carefully with the pattern of areoles in the mature leaf (Figs. 2, 3). The pattern of polygonal areoles constituting the minor venation of the leaf, as exemplified by *Liriodendron*, is of very widespread distribution in angiosperms. It is assumed as a working hypothesis that such a pattern is a basic type from which the distinctive venation



FIGS. 17, 18. Transverse sections of laminae at two stages of development,  $\times 500$ . Figure 17 (above): Original 6-layered condition (between secondary veins) with differentiation of a tertiary in the third layer from the adaxial surface. Figure 18 (below): Several stages in the development of minor veins and the occurrence of periclinal divisions in the various subprotodermal layers. Stippling indicates provascular initials as distinguished from those cells directly above which will become the sclerenchymatous portion of the bundles.

types displayed by the Quinaceae and certain members of the Rubiaceae have evolved by parallel trends toward a lineolate disposition culminating in a lineolate orientation of the entire intercostal venation. The venation of *Bobea* may be considered to be typologically intermediate between that of *Liriodendron* and *Quiina*. It is therefore interesting to note that the ontogeny of the foliar venation presented in this paper also may be regarded as intermediate between the two known extremes. In *Bobea* the first intercostal veins are delimited at a time when the insertion of anticlinal cell walls in the plate meristem which produces procambium for the minor venation is apparently random. The tertiaries and quaternaries thus delimited display a pattern in the



FIGS. 19, 20. Paradermal sections of the same area of a lamina at two levels after the ultimate areoles have been delimited,  $\times 500$ . Figure 19 (left) represents a section  $12\ \mu$  below that in Figure 20, showing a group of areoles enclosed by well-developed procambial strands. The panels of ground tissue will become mesophyll. Figure 20 (right): Same area  $12\ \mu$  higher showing pattern of superimposed sclerenchyma, much more intricate, still actively differentiating apparently.

mature lamina which is not basically different from that of *Liriodendron*. In the intervening areas of the plate meristem concerned there is, then, a decided tendency for groups of elongate cells to be produced by series of similarly oriented cell divisions. From these groups are finally differentiated parallel-oriented minor veins delimiting areoles which are markedly elongate with a tendency to be rectangular rather than polygonal. In this respect, *Bobea* to some extent resembles *Quiina*, in which the entire intercostal venation is derived from a plate of embryonic cells in which the general orientation of elongate cells clearly foreshadows the mature, highly lineolate venation. It is therefore concluded that the ontogenetic sequence in the development of the lamina of *Bobea* represents a divergence in its later aspects toward that of *Quiina* which

correlates with its difference in foliar venation pattern.

A critical evaluation of the above hypothesis must await the results from additional ontogenetic studies. Further investigations in the tribe Guettardeae would be highly rewarding, since in this apparently natural group there is such a wide variety of venation patterns. It is hoped that appropriate material of members of this group can be obtained to augment the present investigation. Similar studies should also be made of other representatives of the *Liriodendron* type of venation to test whether the postulated correlation here presented does indeed exist. In addition to intensive investigation of selected venation types there is also the need for more extensive surveys, such as have been initiated by the author in the Rubiaceae, to further our under-



FIG. 21. Illustrating the general nature in paradermal section of the ground meristem and procambial reticulum at a median phase of leaf development, X 600. Secondary at extreme upper left.

#### SUMMARY

standing of variation in foliar venation and the significance of such variation in systematics.

The foliar venation of *Bobea elatior* has been described. The distinctive feature of the venation pattern is a minor vein system composed of elongate, similarly oriented areoles, usually lacking vein endings. Thus the minor veins produce in some areas of the lamina a lineolate effect. An ontogenetic investigation of the lamina showed that the elongate areoles making up the minor venation are derived by a fairly precise differentiation process from a plate-meristem of markedly elongate cells, whose arrangement clearly foreshadows the

vein pattern of the mature leaf. A comparison is made between the development of the venation of *Bobea* and that of other known types.

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